International Journal of Bio-Technology and Research (IJBTR)
ISSN(P): 2249-6858; ISSN(E): 2249-796X
Vol. 4, Issue 4, Aug 2014, 19-24

ESTIMATION OF MOLECULAR DIVERSITY BETWEEN IMPORTANT NATIVE AND PURE FEMALE ASIATIC CHICKENS USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD)

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ABSTRACT

RAPD was used to determine the molecular genetic diversity of four chicken species viz. White Plymouth Rock – Rhode Island, commercial broiler and Aseel. Using eight oligo primers. a total 68 amplicons (average 8.5per primer) were obtained of which 51 polymorphic. Total 68 banding pattern were observed with an average of 7.6 per primer followed by 0.82% average discriminatory power, and 0.38 average PIC value. Based on UPGMA, Jaccard`s coefficient similarity range from 17 % (RIR2 and CB1) to 87 % (CB1 and CB2) Based on SHAN Cluster algorithms 12 accession grouped in four cluster group, Groups 1 with two accession (RIR2 and WPR2) group 2 with two accession (RIR1 WPR3) group 3 with 6 accession with two sp. Aseel and Commercial Broiler (AS1, AS2, AS3 CB1 CB2 and CB3 and group 4 with 2 accession (WPR1 and WPR2). In this study genetic relationships information generated through RAPD would be useful in utilization for future breeding purpose.

KEYWORDS: Molecular Marker, RAPD, PCR, Genetic Diversity, Poultry, Chicken

INTRODUCTION

During the last three decades several DNA markers such as RAPD, AFLP, RFLP and microsatellites have successfully developed and utilized genetic diversity in chicken studies (Crooijmans *et al*., 1996; Ponsuksili*et al*., 1996; Vanhala *et al*., 1998; van Marle-Köster & Nel, 2000; Weigend & Romanov, 2001; Tadelle, 2003). Microsatellites were also used for commercial as well as rare breeds (Laval *et al.*, 2000**)**. RAPD detecting genetic relationships among chicken populations have been reported by (Sharma *et al*., 2001; Olowofeso *et al.*, 2005). RAPD used single primer of arbitrary nucleotide sequence (Williams *et al*., 1990). The method is also capable of sampling genome randomly unlike the allozymes and the restriction fragment length polymorphisms (RFLPs) (Wang and Dai, 2001). Our objectives were to characterize the different varieties of the chickens through RAPD for future breeding purpose and to identify the banding pattern of primer for identification of chicken species.

MATERIALS AND METHODS

DNA Isolation

The experimental material consist four chicken breeds. Accessions were labeled as WPR 1, WPR2 WPR3, (White Plymouth Rock – female) RIR1, RIR2 and RIR3 (Rhode Island Red–female), CB1, CB2, CB3 (Commercial broiler- female) and AS1, AS2 AS3 (Aseel-female). Collected from INDUS Poultry breeders Hyderabad India and Central Poultry Performance Testing Centre Gurgaon India. Blood was collected in 2 ml tubes containing EDTA, as anticoagulant and stored at –80°C by using the procedure suggested by Hoelzel (1992).

DNA Quantification

0.8 % agarose gel electrophoresis was used to quantify DNA and check the integrity of genomic DNA. All DNA samples were also quantified by spectrophotometer. The measurements were taken at λ260 nm and λ280 nm. The purity of DNA samples ranged from 1.8 up to 1.9. Samples were then diluted to 25 ng/µl for use in RAPD PCR.

Primers and PCR

The primers used were OPA-07, OPA-08, OPA-18, OPB-02, OPE-05, OPM-13, OPO-15, OPT-20 (see Table 2) from (Operon Technologies). The mixture of the PCR reaction had a final volume of 25 µl and contained 50 ng of genomic DNA, 1 × Taq buffer, 1.5 mM MgCl2, 0.2 mM dNTP mix, 20 pmol of each primer,1.00 Unit Taq DNA polymerase ("Fermentas"). The DNA was amplification in a Thermo cycler 'Bio-Rad T100' that was programmed as follows: (95˚C) 3 minutes(denaturation), (32˚C) 30 second (annealing) and (72˚C) 1 minutes(extension) followed by 44 cycle (94˚C) 1 minute (denaturation), (32˚C) 1 minute(annealing), (72˚C) 1 minutes (extension) and final extension (72˚C) 7 minutes (see Figure 5). The PCR products were separated on 1.5% agarose gels in 0.5x TBE buffer stained by ethidium bromide. and photographed by using a Gel Doc System.

Scoring Band Pattern and Statistical Analysis

DNA profiles of 12 chicken accessions were scored as matrix comparison pot in computer program NTsys 2.0 as (0 and 1) for missing and appearing DNA bands to analyze for the distance similarity using Jaccard method (1908), The resulting similarity matrix thus generated was used to construct a phenetic dendrogram using the UPGMA (Unweight Pair-Groups Method using Arithmetic Average) (Sneath and Sokal, 1973) and SAHN clustering analysis. The programs were used to perform a principal coordinate's analysis.

RESULTS AND DISCUSSIONS

Using eight oligo primer total 68 amplicons (average 8.5per / primer) were obtained out of which 51 polymorphic Mini. 4 polymorphic in OPM-13 and 5 in OPE05 and Mixi. 8 were OPA18 and OPT20 Followed by 0.82% average discriminatory power, Mini. 6 amplicons in OPM-13 and OPE-5 and maxi. 11 amplicons were OPA -07. Total 61 banding pattern were observed with an average of 7.6 per primer. Mini. Banding pattern were 5 in OPM-13 and followed by 6 patterns with OPB-02 and OPB-20. Maxi. Banding patter were 10 with OPA-08 and OPA-15. and 0.38 average PIC value mini0.30 in OPT-20 and maxi 0.44 in OPM-13. Molecular genetic diversity of four chicken species viz. White Plymouth Rock – Rhode Island, Commercial broiler and Aseel establish with RAPD were within sp. similarity in WPR group have minimum 35% between WPR1 and WPR3 and maximum 46 % between WPR1and WPR2. Group RIR have minimum 21% and maximum 34% similarly groups CB having minimum 77% and maximum 87% and Groups Aseel having minimum 57% and maximum 77%. Between species groups genetic similarities were WPR and RIR Mini. 25% and Max. 59 %, Group WPR and CB minimum 28 and Maximum 51%. Groups WPR and AS Mini. 20 % and maximum 47 %. Group RIR and CB Mini. 17 % and Max. 36. Group RIR and AS Mini. 21 % and Maxi 43% and Group CB and AS Mini. 50% and Maxi. 73%. Based on UPGMA, Jaccard`s coefficient similarity range from mini. 17% (RIR2 and CB1) to maxi. 87 % (CB1 and CB2) Based on SHAN Cluster algorithms 12 accession grouped in four cluster group, Groups 1 with two accession (RIR2 and WPR2) groups 2 with two accession (RIR1 WPR3) groups 3 with 6 accession with two sp. Aseel and Commercial Boiler (AS1,AS2,AS3 CB1 CB2 and CB3 and groups 4 with 2 accession (WPR1 and WPR2).

CONCLUSIONS

In this study genetic relationships information generated through RAPD by selecting the primers with high discriminatory power would be useful for utilization for future breeding purpose.

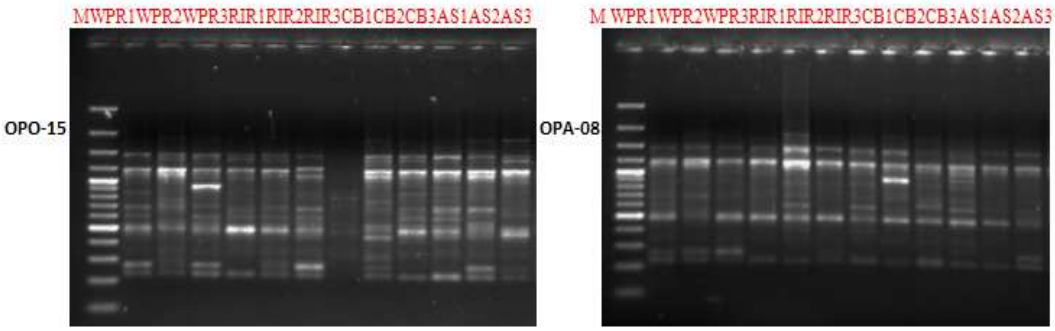

Figure 1: RAPD Amplification Generated for 12 Accession (WPR), (RIR), (CB) and (AS) by Primer OPO-15 and OPA-08

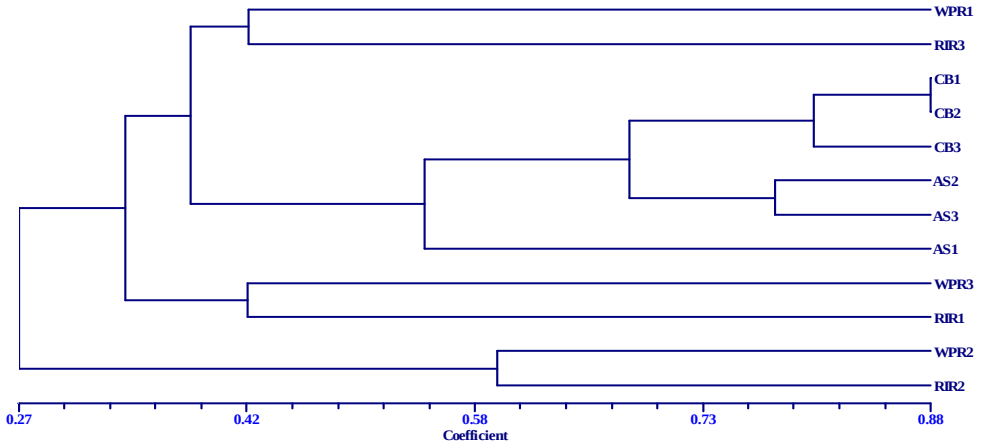

Figure 2: Dendogram Showing Relationship among Twelve Chicken Accessions Generated by UPGMA Analysis Based on Single Primers

	WPR1											
WPR1	1.00	WPR2										
WPR2	0.46	1.00	WPR3									
WPR3	0.35	0.39	1.00	RIR1								
RIR1	0.31	0.25	0.42	1.00	RIR2							
RIR2	0.28	0.59	0.26	0.34	1.00	RIR3						
RIR3	0.42	0.33	0.35	0.26	0.21	1.00	CB1					
CB1	0.44	0.31	0.41	0.20	0.17	0.36	1.00	CB2				
CB2	0.51	0.32	0.44	0.27	0.18	0.36	0.87	1.00	CB3			
CB3	0.40	0.28	0.51	0.33	0.21	0.28	0.77	0.82	1.00	AS1		
AS1	0.27	0.21	0.31	0.31	0.24	0.28	0.50	0.50	0.50	1.00	AS2	
AS2	0.41	0.20	0.38	0.34	0.21	0.37	0.67	0.72	0.70	0.63	1.00	AS3
AS3	0.47	0.30	0.34	0.30	0.21	0.43	0.62	0.73	0.60	0.57	0.77	1.00

Figure 3: Jaccard's Similarity Coefficient Developed from 8 Oligo Primer Amplicons Generated amongst the 12 Chicken Accessions

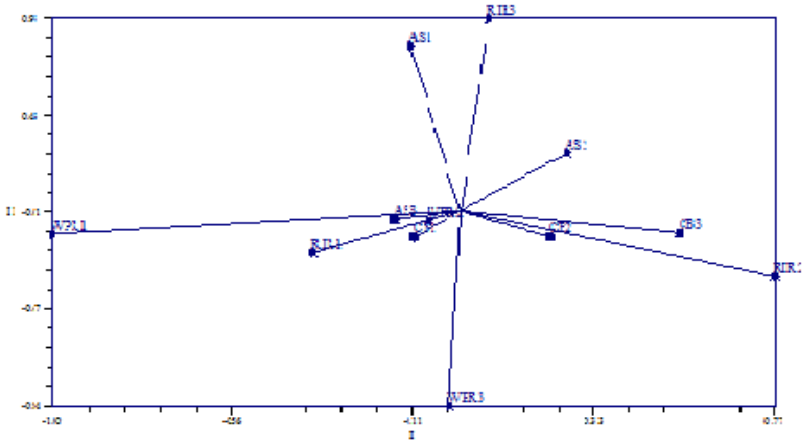

Figure 4: Principle Coordinates Analysis of Using RAPD Profile of 8 Oligo Decamer Primer for 12 Chicken Accession

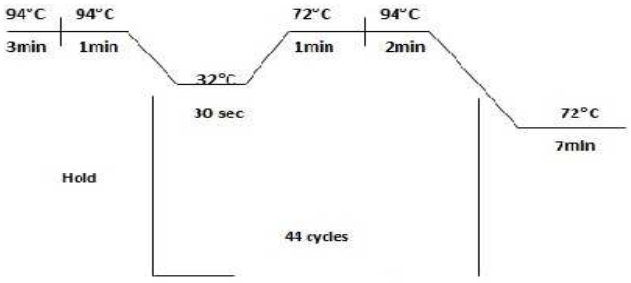

Figure 5: Steps in PCR Amplification

Table 1: Within and between Group Genetic Similarity vs. Diversity

Within Group Genetic Similarity		
Name of Groups	**Similarity (%) (Mini/Maxi)**	**Accession**
WPR	35	WPR1 and WPR3
	46	WPR1 and WPR2
RIR	21	RIR2and RIR3
	34	RIR1and RIR2
CB	77	CB1and CB3
	87	CB1 and CB2
AS	57	AS1 and AS3
	77	AS2 and AS3
Between Groups' Genetic Similarity		
Name of Groups	**Similarity (%) (Mini/Maxi)**	**Accession**
WPR and RIR	25	WPR2 and RIR1
	59	WPR2 and RIR2
WPR and CB	28	WPR2and CB3
	51	WPR1 and CB2 WPR3 and CB3
WPR and AS	20	WPR2 and AS2
	47	WPR1and AS3
RIR and CB	17	RIR2 and CB1

	Table 1: Contd.,	
	36	RIR3 and CB1 RIR3 and CB2
RIR and AS	21	RIR2and AS2 RIR2 and AS3
	43	RIR3 and AS3
CB and AS	50	CB1 and AS1 CB2 and AS1 CB3and AS1
	73	CB2and AS3

Table 2: Detail of Primer Sequences, Melting Temperatures (TM), GC Content, Number Amplicons, Polymorphic Amplicons, Primer Discriminatory Power Polymorphic Information Content (PIC) Developed through the RAPD-PCR of 20 Chicken

Primer Name	Primer Sequence	TM (˚C)	GC (%)	Number Total of Band	Polymorphic Band	Banding Pattern	Discriminatory Power	PIC Value
OPA-07	GAAACGGGTG	32	60%	11	7	9	0.89	0.43
OPA-08	GTGACGTAGG	32	60%	9	6	10	0.91	0.42
OPA-18	AGGTGACCGT	32	60%	10	8	8	0.82	0.40
OPB-02	TGATCCCTGG	32	60%	7	6	6	0.71	0.36
OPE-05	TCAGGGAGGT	32	60%	6	5	7	0.81	0.38
OPM-13	GGTGGTCAAG	32	60%	6	4	5	0.68	0.44
OPO-15	TGGCGTCCTT	32	60%	10	7	10	0.91	0.36
OPT-20	GACCAATGCC	32	60%	9	8	6	0.85	0.30
Average				8.5	6.3	7.6	0.82	0.38

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